# 39th Plant Development Workshop

## University of Guelph

### Saturday Nov 19, 2005

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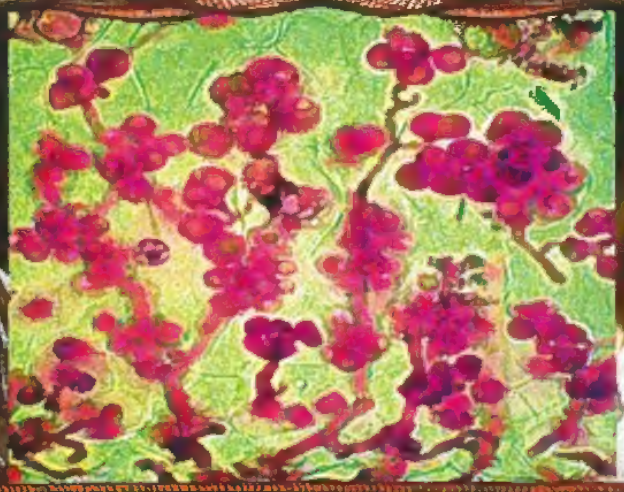

# 39th Plant Development Workshop

## Saturday, November 19, 2005
## University of Guelph

Schedule:

8:00-8:50 Registration/Coffee

8:50-9:00 Welcome: Usher Posluszny

9:00-9:30 **'Morpho'-EVO-DEVO: the plant morphology context**
Christian Lacroix

9:30-9:45 **BLADE-ON-PETIOLE-dependent signaling: new insights into mechanisms of leaf and floral patterning in *Arabidopsis***
Shelley Hepworth, Yeulin Zhang, Jon Taylor, Xin Li, and George Haughn

9:45-10:00 **TCP15: A Regulator of Lateral Branching in *Arabidopsis thaliana***
Rashida Patel and Dan Riggs

10:00-10:15 **Phylogenetic Trends and Comparative Ontogeny in the Grape Family**
Susan Timmons, Usher Posluszny, and Jean Gerrath

10:15-10:30 **The mode of leaflet separation in the Parlour Palm.**
Julia Kuzmina, Nancy G. Dengler, and Usher Posluszny

10:30-11:00 Coffee Break and Posters

11:00-11:15 **Starches and their Functional Properties**
Ian J. Tetlow

11:15-11:30 **Enzymes involved in Cereal Starch Biosynthesis**
Nicole Bresolin, Ian Tetlow, Michael Emes

11:30-11:45 **Mechanisms for the control of wheat endosperm ADP-glucose pyrophosphorylase**
Mark Burrell, Ian Tetlow and Michael Emes

11:45-12:00 **Evidence for Protein-Protein Interactions between Enzymes of Starch Biosynthesis.**
Fushan Liu, Ian Tetlow and Mike Emes

12:00-12:15 **Leaf anatomy and intercellular enzyme compartmentation in $C_3$-$C_4$ intermediate species in the genus *Heliotropium* L. (Boraginaceae)**
Riyadh Muhaidat, Nancy Dengler and Rowan Sage

12:15-12:30 **Multiple origins of intermediate photosynthesis in *Flaveria***
Athena D. McKown and Nancy Dengler

12:30-2:00 Lunch in the University Centre. Posters in the lobby of the Thornborough Lecture Hall

2:00-2:15 **A MYB transcription factor at the interface of nutrient perception and phenotypic response.**
Malcolm M. Campbell

2:15-2:30 **Characterization of Age-Related Resistance Mutants in *Arabidopsis.***
Jessie Carviel, Asif Mohammad, Fadi Al-daoud, and Robin K. Cameron

2:30-2:45 **Tomato eIF5A-3 regulates tolerance to pathogen attack**
Fengshan Ma, Zhongda (Chris) Liu, Tzann-Wei (Mike) Wang, Linda McNamara, John E. Thompson

2:45-3:00 **Screen for Suppressors of Hypernodulation: New Alleles, and Novel Loci**
Jeremy Murray, B. Karas, S. Sato, S. Tabata, M. Parniske, S. Radutoiu, J. Perry, R. Geil, C. Wagg, L. Amyot, L. Ross, J. Stougaard, L. Peterson, and K. Szczyglowski.

3:00-3:15 **Invasion of *Lotus japonicus root hairless 1* by *Mesorhizobium loti* Involves the Nodulation Factor – Dependent Induction of Root Hairs.**
Bogumil Karas, Jeremy Murray, Monika Gorzelak, Alexandra Smith, Shusei Sato, Satoshi Tabata and Krzysztof Szczyglowski

3:15-3:45 Break…you could get coffee or juice at the University centre

3:45-4:00 **Developmental morphology of several vine members of the Cucurbitaceae**
Tim Zitnak and Usher Posluszny

4:00-4:15 **Modifying substances in soybean root epidermal walls: relation to pathogen resistance**
Xingxiao Fang, Raymond Thomas , Carol A. Peterson, Mark Bernards, Mark Gijzen

4:15-4:30 **Water movement through seed coats and into embryos of permeable and stone seeds of soybean**
Chris J. Meyer, Ernst Steudle, Carol A. Peterson

4:30-4:45 **Role of sclereids in metal detoxification of *Eriophorum vaginatum* L.**
Sarah J. Bogart and Ewa Cholewa

5:00-6:30 Social get-together at the Grad Lounge, University Centre 5th Floor.....beer, wine, cheese and nibbles.

# Abstracts for the 39th Plant Development Workshop

**Oral Presentations:**

### 'Morpho'-EVO-DEVO: the plant morphology context

Christian Lacroix
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Classical plant morphology and dynamic morphology represent two theoretical frameworks used to describe and understand the bauplan 'body plan' of vascular plants, especially flowering plants. Generally, plants are interpreted in terms of three mutually exclusive structural categories: stem, leaf, and root. Stem and leaf together constitute a shoot which usually shows axillary branching. According to the classical approach, organ identity can be predicted by its relative position within the plant's bauplan. This model applies to many but not all flowering plants. There are groups with forms that do not clearly fit into the classical model. In these cases, a dynamic morphological perspective may serve as a more encompassing model. It accepts developmental mosaics between stem, leaf, and root. This continuum model was revived during the pre 'EVO-DEVO' period by Agnes Arber, Rolf Sattler, and others. Some of the ideas of this dynamic approach are compatible with results obtained by evolutionary developmental plant biologists.

### BLADE-ON-PETIOLE-dependent signaling: new insights into mechanisms of leaf and floral patterning in *Arabidopsis*

Shelley Hepworth[1], Yeulin Zhang[2], Jon Taylor[2], Xin Li[2], and George Haughn[2]
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e-mail: shelley_hepworth@carleton.ca

A remarkable degree of architectural diversity exists between plant species. An important goal of plant developmental biologists has been to identify genes that control patterning and to understand how these genes sculpt plant organs. We have recently identified a role for two potentially redox-regulated signaling proteins, designated BLADE-ON-PETIOLE (BOP) 1 and 2, in controlling growth asymmetry, a crucial aspect of patterning in plant morphogenesis. Simultaneous disruption of the *BOP* genes leads to patterning changes associated with mis-regulation of growth along axes of bilateral symmetry (mirror image symmetry) in leaves and flowers. Phenotypes in the double mutant, which include leafy petioles, lack of floral-organ abscission, and bract-bearing asymmetric flowers, correlate closely with the expression pattern of the *BOP* genes. We provide evidence that the BOP proteins act by modulating the activity of TGA

transcription factors, in much the same way as previously shown for the related protein NPR1, a positive regulator of plant disease resistance. Our data indicate that the BOP proteins function in an as-yet-uncharacterized NPR1-like signaling pathway that serves to integrate spatial information with growth asymmetry and thus plays a key role in axial patterning of plant organs.

**TCP15: A Regulator of Lateral Branching in *Arabidopsis thaliana***

Rashida Patel and Dan Riggs
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TCP genes encode a class of plant specific transcription factors that contribute to governing a variety of aspects of plant development, including leaf curvature, cell proliferation, floral organ asymmetry, and lateral branching. This class of proteins is named after the three founding members; *teosinte branched1* found in maize, *cycloidea* found in *Antirrhinum*, and the PCF proteins found in rice. Multiple members of this family exist in different plant species, with 24 TCP encoding genes present in the *Arabidopsis* genome. Members of this family contain a conserved motif dubbed the TCP domain, which is responsible for DNA binding and regulation of target gene expression. To identify candidate factors that may regulate lateral branching in Arabidopsis, an enhancer trap library screen for lines exhibiting nodal expression patterns was performed. One such line was found and subsequently was identified as TCP15. BLAST homology search revealed that this gene is similar to a cotton expressed sequence tag encoding an auxin induced basic helix-loop-helix transcription factor. Expression of TCP15 was examined using this enhancer trap line and found to have an intriguing pattern that mirrors that of the auxin reporter DR5::GUS. Preliminary results suggest that this gene is not directly modulated in expression by auxin, however its regulation by the hormone cytokinin, another hormone influencing axillary branching remains to be explored. While homozygous TCP15 null mutants had no apparent phenotype, four independent TCP15 overexpressing lines show various degrees of loss of apical dominance and increased lateral branching in addition to 'aerial rosette' cluster formation at nodes. The high degree of TCP gene redundancy in *Arabidopsis* may account for the lack of an observable phenotype in the single mutant. Thus it is probable that the TCP15 protein may be a factor responsible for regulating axillary branching, by promoting growth of intercalary meristems.

**Phylogenetic Trends and Comparative Ontogeny in the Grape Family**

Susan Timmons, Usher Posluszny, and Jean Gerrath
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The grape family (Vitaceae) is of great economic and ecological importance; however its phylogeny remains unclear. Recent phylogenies using nuclear *ITS1* and plastid *trnL* DNA have disagreed with traditional grouping of species by placing *Vitis* species with *Cissus* species and linking *V. rotundifolia* (muscadine grape) more closely with these *Cissus* species than with other *Vitis* species. This study used comparative developmental morphology as an independent method to investigate vitaceous phylogeny. Ontogeny of vegetative and floral structures of *V. rotundifolia* was studied using epi-illumination microscopy and histology. These results were compared with those from similar studies of *Vitis* cultivar 'Ventura', *V. riparia*, and *C. antarctica*. With the exception of stipule development and the timing of axillary bud initiation, vegetative and floral characters of *V. rotundifolia* were most similar to those of *V. riparia*. The presence of hydathodes, calyptra, and ring-shaped gynoecial disk, along with anther development, were common in all *Vitis* species studied. These results suggest *V. rotundifolia* is more developmentally similar to other *Vitis* species than with *C. antarctica*, supporting traditional classifications and contrary to recent *ITS1* and *trnL* DNA phylogenies.

**The mode of leaflet separation in the Parlour Palm.**

Julia Kuzmina, Nancy G. Dengler, and Usher Posluszny
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Leaflet development in palms is unique and unlike any observed in other angiosperms. It involves two steps: 1) plication formation and 2) leaflet separation. Plication formation was shown to occur through differential growth of the primordial tissue in a series of papers by Kaplan et al. (1982a and b) and Dengler et al. (1982). In all of the previous literature on the subject of palm leaf formation, the process of abscission has been described as the cause for leaflet separation. Abscission seems to have been accepted as a fact but has not been shown explicitly to be the primary processes involved in leaflet separation. Thus, if there is the occurrence of schizogeny followed by either cell death or formation of a protective layer or both in palm leaves, then it can be concluded that abscission is in fact involved in the process of leaflet separation. The objective of this study is to find when and how leaflet separation occurs in the parlour palm (*Chamaedorea elegans*) using techniques such as scanning electron microscopy, transmission electron microscopy, histochemistry and immunolocalization, which were not available to the previous researchers in this subject. Our work to date has shown that schizogeny does take place in *Chamaedorea elegans*, but the presence of cell death as well as the formation of a protective layer has yet to be determined through further research.

**Starches and their Functional Properties**

Ian J. Tetlow
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Starch is a major component of the human diet and is also used in a wide variety of industrial and non-food applications. Variations in polymer composition and fine structure underpin many of these end-uses. The structural properties of starches exploited in certain industrial and food applications will be discussed, with particular emphasis on the human health benefits associated with resistant starches in the diet.

**Enzymes involved in Cereal Starch Biosynthesis**

Nicole Bresolin, Ian Tetlow, Michael Emes
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Starch biosynthesis occurs in the plastids of plants. In cereal crops a large amount of storage starch is produced within the amyloplasts of developing seeds. Starch biosynthesis requires the concerted action of four different classes of enzymes. ADP-glucose pyrophosphorylase (AGPase: EC 2.7.7.27) is regarded as the first committed step in the pathway producing the activated glucosyl precursor (ADP-glucose) which is the source of glucose for chain elongation and subsequent branching. Starch synthases (SS: EC 2.4.1.21) catalyse the elongation of the chains, granule bound starch synthase (GBSS) is involved in the synthesis of amylose, while the soluble starch synthases elongate glucan chains of amylopectin. Branch points are introduced by the action of branching enzymes (BE: EC 2.4.1.18). Genetic evidence demonstrates that the debranching enzymes isoamylase (EC 3.2.1.68) and pullulanase (EC 3.2.1.41) also play a role in starch synthesis. The characterisation of these proteins has been aided by the identification of mutants lacking the function of one or more of these genes. The role of these enzymes in the formation of starch in higher plants will be discussed.

**Mechanisms for the control of wheat endosperm ADP-glucose pyrophosphorylase**

Mark Burrell, Ian Tetlow and Michael Emes
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Starch is a highly important plant product that has a large variety of different usages in both the food industry and within industry itself. The production of starch occurs within plastids of all the higher plants. The first step in making starch is the conversion of the soluble sugar glucose 1-phosphate (G1P) into ADP-glucose (ADPGlc) by the action of the enzyme ADP-glucose pyrophosphorylase (AGPase). AGPase is the first committed step in starch biosynthesis. In order to effectively control carbon partitioning within the plant, regulation of AGPase activity is required. There are several different mechanisms by which AGPase is controlled. In many species, the allosteric effectors 3PGA and Pi act to control activity and it is thought that the ratio of these two effectors controls AGPase activity rather than it being the effect of one by itself. In several plants including potato, spinach and wheat, AGPase activity can be controlled by redox modulation and this appears to have a much greater role in controlling AGPase than allosteric effectors. Different mechanisms operate to adjust redox modulation, including light and sugar levels. The role of these different mechanisms for the control of AGPase in wheat endosperm will be discussed.

**Evidence for Protein-Protein Interactions between Enzymes of Starch Biosynthesis.**

Fushan Liu, Ian Tetlow, Mike Emes
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Starch provides a major caloric source for the human population and is also an important industrial commodity. Although the pathway of starch biosynthesis is not completely understood, it is known to involve at least four groups of committed enzymes: ADP glucose pyrophosphorylase (AGPase), starch synthases (SSs), starch branching enzymes (SBEs), debranching enzymes (DBEs). SSs add glucosyl units to the non-reducing end of a glucan chain through α (1-4) linkages, thus elongating the linear chains, whilst SBEs introduce the α (1-6) linkages linkages found in amylopectin. Recent research involving cross linking and co-immunoprecipitation experiments indicated that forms of SS and SBE are able to form functional interactions. The precise role of the SBE/SS protein complexes is unclear, but these protein assemblies may facilitate substrate channeling as the product of one reaction becomes the substrate for another enzyme in the complex. Evidence is also presented which indicates that certain SBE/SS protein complexes are formed at specific times during endosperm development.

**Leaf anatomy and intercellular enzyme compartmentation in $C_3$-$C_4$ intermediates species in the genus *Heliotropium* L. (Boraginaceae)**

Riyadh Muhaidat, Nancy Dengler and Rowan Sage.
Department of Botany, University of Toronto

*Heliotropium* section *Orthostachys* contains dozens of $C_3$ species and $C_4$ species. Given this diversity of $C_3$ and $C_4$ species in a single genus, we investigated the possibility that some *Heliotropium* species are intermediate between $C_3$ and $C_4$ photosynthesis. Leaf anatomy, photosynthetic $CO_2$ compensation points, and intercellular distribution of Rubisco and glycine decarboxylase were analyzed for seven species of the genus, namely *H. tenellum*, *H. procumbens*, *H. convulvulaceum*, *H. greggii*. *H. texanum* and *H. polyphyllum*. Leaves of *H. tenellum* exhibit an internal architecture typical of $C_3$ dicotyledonous leaf anatomy. *H. procumbens* demonstrates a weakly developed *Kranz* leaf anatomy, while *H. convolvulaceum* and *H. greggii* possess a distinctive *Kranz*-like leaf system, but less differentiated than the fully developed *Kranz* pattern in *H. texanum* and *H. polyphyllum*. Photosynthetic $CO_2$ compensation points of *H. tenellum* and *H. procumbens* are very high within the range typical of $C_3$ plants, but slightly lower in *H. procumbens*. The points were very low in *H. convolvulaceum* and *H. greggii* relative to those of $C_3$ plants, but still higher than in the $C_4$ species *H. texanum* and *H. polyphyllum*. Immunolabeling experiments revealed that Rubisco is distributed throughout leaf chlorenchyma in *H. tenellum* and *H. procumbens*, is totally confined to bundle sheath cells in the $C_4$ species, and has intermediate distribution in *H. convulvulaceum* and *H. greggii*. For glycine decarboxylase, labeling occurs in all photosynthetic cells of the $C_3$ species, but is exclusively concentrated in bundle sheath cells in the leaves of $C_3$-$C_4$ and $C_4$ species. We conclude that *H. tenellum* is a strictly a $C_3$ plant, whereas *H. procumbens*, *H. convolvulaceum* and *H. greggii* are true $C_3$-$C_4$ intermediate heliotropes, and represent an early phase of $C_3$-$C_4$ intermediacy evolution . Notably, the appearance of *Kranz*-like anatomy accompanied by differential compartmentation of photorespiration is consistent with the hypothesis that enlargement of the bundle sheath tissue and an increase in its metabolic activity are critical initial steps in the evolution of the $C_4$ pathway.

**Multiple origins of intermediate photosynthesis in *Flaveria***

Athena D. McKown and Nancy G. Dengler,
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While much is understood about the functioning of the C4 photosynthetic pathway, its evolution remains largely unknown. In some genera, certain species persist as biochemical intermediates between C3 and C4 photosynthesis. These anomalies are thought to represent evolutionary intermediates (i.e. transitional

stages in evolution of the C4 syndrome); however, these species may also represent alternative photosynthetic pathways to C3 and C4 photosynthesis. Within the genus *Flaveria*, there are numerous photosynthetic intermediate species classified as C3-C4 and C4-like. Based on recent phylogenetic work using three gene markers (two nuclear and one chloroplast), these intermediate species are found scattered throughout the phylogenetic tree and indicate parallel evolution of intermediacy within the genus. Some of these species represent evolutionary intermediates, whereas others are found at the tips of the tree and may represent stable endpoints within themselves. This study presents a comparison of the anatomical characters associated with functional C4 photosynthesis, such as Kranz anatomy and vein pattern, with those that have evolved in parallel within clades of intermediates, to determine how parallel evolution may have occurred with the genus *Flaveria*.

**A MYB transcription factor at the interface of nutrient perception and phenotypic response.**

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In plants, nutrients function as critical signals to mediate growth, development and metabolism. The components of the signaling pathways involved in nutrient perception are just beginning to be elucidated. This seminar will present evidence in support of the hypothesis that a member of the *Arabidopsis thaliana* R2R3-MYB family, *At*MYB61, functions to condition the perception of nutritional signals derived from photosynthesis and photorespiration, and that *At*MYB61 shapes plant growth and development in response to these signals. *AtMYB61* expression is induced by sucrose and repressed by a combination of glutamate and glycine, through a pathway that does not appear to involve direct signalling from hexokinase. In keeping with its role as a component of a sugar signalling pathway, *At*MYB61 conditions sugar responsiveness in key aspects of plant development and metabolism, including seed germination, seedling establishment, the formation of vegetative tissues, flowering time, and the accumulation of chlorophyll and anthocyanins. Moreover, *At*MYB61 links the control of carbon acquisition for photosynthate production with the allocation of carbon in sinks such as secondary xylem. *At*MYB61 accomplishes this by modulating transcript abundance of specific sugar-responsive genes. These genes contain a motif in their upstream regulatory regions that is bound by *At*MYB61, and *At*MYB61 activates transcription from this same motif. Thus, *At*MYB61 is a nutrient-response regulator that plays a central role in conditioning disparate, yet fundamentally important adjustments in plant growth, development and metabolism.

References:

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Dubos C, *et al.* (2005) The Plant Journal 43: 348–355

Liang Y-K, Dubos C, *et al.* (2005) Current Biology 15: 1201-1206
Dubos C, Poole M, Ferber E, Evans JH, Dilton-Hill F, Burrill C, Smith R, Newman LJ, Patzlaff A, Surman C, Campbell MM (*submitted*)

**Characterization of Age-Related Resistance Mutants in Arabidopsis**

Jessie Carviel, Asif Mohammad, Fadi Al-daoud, and Robin K. Cameron
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Many plant species possess defense pathways such as Age-Related Resistance ARR. ARR has been observed in numerous plant species, such that disease resistance increases as the plant matures. *Arabidopsis* mutants which do not display ARR are currently being used as tools in an attempt to elucidate the ARR pathway. Mutants defective in the ARR pathway were obtained through classical genetic screening (p17). Additionally, eight T-DNA insertion mutants in genes highly up-regulated during ARR were identified. These mutants were previously assayed for their ability to exhibit ARR by challenging with virulent *Psedomonas syringe pv tomato* and quantitating bacterial growth *in planta* at young and mature time points. *P17* mutants were ARR-defective, implying that the wild type *P17* gene is important for ARR function. The eight T-DNA insertion mutants are currently being retested for their ability to display ARR while the *p17* mutant was further tested to determine if basal resistance, (early disease resistance seen in young plants,) was also affected by the mutation. Young *p17* plants displayed a normal response to *Pst*, suggesting that the mutation is not involved in this pathway. Salicylic acid (SA) is thought to be one of the end products of the ARR pathway and may contribute to ARR as an anti-microbial agent. The intercellular addition of SA to the *p17* mutants improved resistance by two-fold, implying that SA accumulation has been impaired by this mutation and that the wild type *p17* lies upstream of SA in the ARR signaling pathway. We are in the process of mapping *p17* using cleaved amplified polymorphic sequences. Identification of *p17* and the function of the genes isolated in the microarray experiment will provide important insights into the ARR defense pathway.

**Tomato eIF5A-3 regulates tolerance to pathogen attack**

Fengshan Ma, Zhongda (Chris) Liu, Tzann-Wei (Mike) Wang, Linda McNamara, John E. Thompson
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The eukaryotic translation initiation factor 5A (eIF5A), which is a small protein (~18 kDa), is thought to exist ubiquitously in all eukaryotes (eIF5A) and Archaea (aIF5A). eIF5A is post-translationally activated by converting a conserved lysine residue near its N-terminus to hypusine, which is found only in eIF5A. There is evidence that eIF-5A may facilitate translation by shuttling specific mRNAs from

the nucleus to the cytoplasm. In each of the few plant species examined, eIF5A exists in three (*Arabidopsis*) or four isoforms (other plants) that have different functions in a plant's life. For example, one of the four eIF5A isoforms, eIF5A-3, in tomato regulates responses to wounding or pathogen attack. Earlier, we documented that eIF5A-3 is up-regulated in wounded tissues. It is possible to enhance the resistance of tomato plants to pathogens by down-regulating eIF5A-3 expression. In one study, we generated tomato lines that constitutively over-express the 3' UTR of eIF5A-3 in the antisense orientation. These plants exhibited tolerance against *Pseudomonas syringea* to various degrees. There is a correlation between the extent of eIF5A down-regulation and tolerance to the pathogen. This feature is accompanied by changed morphology of the plants and their wound-healing process. These "side effects" probably reflect down-regulation of other eIF5A isoforms. To down-regulate eIF5A-3 with higher specificity, we have taken RNAi and siRNA approaches by targeting short regions of the 3' UTR of eIF5A-3. Transgenic lines have been established and detailed phenotyping and pathological studies will be carried out.

**Screen for Suppressors of Hypernodulation: New Alleles, and Novel Loci**

Murray, J., Karas, B., Sato, S., and Tabata, S., Parniske, M., Radutoiu, S., Perry, J., Geil, R., Wagg, C., Amyot, L., Ross, L., Stougaard, J., Peterson, L., Szczyglowski, K.
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A genetic screen was carried out in the *Lotus japonicus* symbiotic mutant *har1* in an effort to identify mutants that suppressed the hypernodulation phenotype it exhibits in response to infection by *Mesorhizobium loti*. In total 63 mutant lines were isolated. A subset of 33 mutant lines deficient in their ability to nodulate was identified and their corresponding loci were genetically positioned and found to represent a minimum of 10 independent loci. Using a combination of TILLING, sequencing, and mycorrhizal phenotypes, novel alleles of *NIN*, *NFR5*, and *POLLUX* were identified. The remaining 30 mutant lines were able to form nodules but to a lesser degree than *har1*. One of these lines, HIT (hyperinfected), was further characterized. Symbiotic phenotypes of this line include delayed onset of cortical cell divisions, hyperinfection, and abnormal nodule development resulting in deformed nodules that are eventually capable of fixing nitrogen. To facilitate mapping of the low nodulating mutants, an introgression line in which the *har1-1* allele was introduced into a polymorphic background was created.

**Invasion of *Lotus japonicus root hairless 1* by *Mesorhizobium loti* Involves the Nodulation Factor – Dependent Induction of Root Hairs.**

Bogumil Karas[1], Jeremy Murray[1], Monika Gorzelak[2], Alexandra Smith[2], Shusei Sato[3], Satoshi Tabata[3] and Krzysztof Szczyglowski[1*]
[1]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, Ontario, Canada N5V 4T3 (B.K., J.M., K.S.); [2]Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1 (M.G., A.S.); and [3]Kazusa DNA Research Institute, Kisarazu, Chiba, 292–0812, Japan (S.S., S.T.)

In many legumes, including *Lotus japonicus* and *Medicago truncatula*, susceptible root hairs are the primary sites for the initial signal perception and physical contact between the host plant and the compatible nitrogen-fixing bacteria that leads to the initiation of root invasion and nodule organogenesis. However, diverse mechanisms of nodulation have been described in a variety of legume species that do not rely on root hairs. To clarify the significance of root hairs during the *L. japonicus-Mesorhizobium loti* symbiosis, we have isolated and performed a detailed analysis of four independent *L. japonicus* root hair developmental mutants. We show that although important for the efficient colonization of roots, the presence of wild-type root hairs is not required for the initiation of nodule primordia (NP) organogenesis and the colonization of the nodule structures. In the genetic background of the *L. japonicus root hairless 1* mutant, the nodulation factor-dependent formation of NP provides the structural basis for alternative modes of invasion by *M. loti*. Surprisingly, one mode of root colonization involves nodulation factor-dependent induction of NP-associated cortical root hairs and epidermal root hairs, which, in turn, support bacterial invasion. In addition, entry of *M. loti* through cracks at the cortical surface of the NP has been observed. These novel mechanisms of nodule colonization by *M. loti* explain the fully functional, albeit significantly delayed, nodulation phenotype of the *L. japonicus ROOT HAIRLESS* mutant.

**Developmental morphology of several vine members of the Cucurbitaceae**

Tim Zitnak and Usher Posluszny
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Apical meristem development and subsequent branching of many Cucurbitaceae species is of interest due to the complex nature of the node; however few ontogeny studies exist to provide a morphological framework for investigation. This study provides a set of morphological characteristics by examining the ontogeny of several species of vine-forming Cucurbitaceae with similar growth habits but different tendril architecture. The floral development and phyllotactic patterns of the apical complex and shoot architecture were examined. Early ontogeny of the apical complex was determined using epi-illumination light microscopy. Results for *Sicyos angulatus* and *Ecballium elaterium* (L.) were

compared to a similar study of *Echinocystis lobata* (Michaud). For all species examined, each leaf primordium has a complex axillary structure, offset from the leaf axil, which is not merely an axillary bud. This axillary complex undergoes a series of asymmetric divisions which give rise to structures in a set physical sequence: a male inflorescence, a female inflorescence, an axillary bud and a tendril but *E. elaterium* lacks the tendril. The axillary bud does not stay dormant and develops into either a compressed, quiescent shoot or continues growth to produce a branch. *S. angulatus* and *E. lobata* have the same physical sequence of axillary structures but division timing differs. The timing of *E. elaterium* has not yet been determined. This suggests Cucurbitaceae vines develop from an axillary complex with the same physical sequence of axillary structures.

**Modifying substances in soybean root epidermal walls: relation to pathogen resistance**

Xingxiao Fang[1], Raymond Thomas[2], Carol A. Peterson[1], Mark Bernards[2], Mark Gijzen[3]
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Epidermal walls in roots are generally modified, as shown in previous work. What is the chemical nature of this modification, and does it play a role in pathogen resistance? To answer these questions, soybean (*Glycine max*) was used as a test species. The root epidermal wall compositions of two cultivars were compared; one (Conrad) is resistant to *Phytophthora sojae* and the other (OX 760-6) is susceptible to this root-rot oomycete. Epidermal walls of both cultivars were proved to contain suberin but the resistant cultivar had a greater quantity of both the aliphatic and aromatic components of the polymer than the susceptible cultivar. After attacked by zoospores of *P. sojae*, roots of the resistant cultivar had fewer internal haustoria than those of the susceptible cultivar. It was not possible to differentiate the hyphal walls of the oomycete from the cell walls of the root. However, germ tubes and haustoria could be identified by staining the "halo" of callose, at the point of entry into the cells and in the interior of the infected cells, respectively, with aniline blue. In both cases, the cortical cells near the epidermis of the infected roots reacted to the pathogen by suberizing their walls. Resistant roots browned whereas susceptible roots remained light. It is concluded that resistant roots react more strongly and efficiently to pathogen invasion than susceptible roots.

**Water movement through seed coats and into embryos of permeable and stone seeds of soybean**

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Usually, when soybean seeds are immersed in water, they soon begin to imbibe, soften and swell. Stone seeds, however, can remain dry and hard for long periods of time. Previously, a correlation was noted between the structure of the outer cuticle of the seed and its capacity for water uptake: the cuticle of permeable seeds had small cracks whereas that of stone seeds was continuous. The present investigation confirmed that the stone seeded trait is a feature of the seed coat, since isolated embryos from both stone and permeable seeds took up water at equal rates. When hydrated, seed coats of stone seeds were permeable to water but their hydraulic conductivity was smaller than that of coats from permeable seeds by a factor of five. Hydrated seed coats of both permeable and stone seeds show weak osmometer properties. Whole, permeable seeds take up water initially through the dorsal side of the seed coat and later through the hilum. The sites of initial water uptake by the cotyledons correlate with externally visible changes (i.e. wrinkling) in the coat. Some circumferential movement of water through the coat occurs, presumably through the air spaces of the osteosclereid layer. Imbibition of whole seeds is a two-phase process, the first dominated by hydration of the seed coat and the second by hydration of the embryo.

**Role of sclereids in metal detoxification of *Eriophorum vaginatum* L.**

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A recent study suggested the survival of *Eriophorum vaginatum* L., a tussock forming sedge, might be tied to the presence of highly lignified sclerenchyma cells, or sclereids. *E. vaginatum* thrives in cold, nutrient poor environments, and metal contaminated industrial areas where previous studies have shown it to accumulate and store metals such as $^{137}Cs$, Pb, Cu, and As. Sclereids of this species are associated with vascular bundles and may facilitate internal nutrient recycling within its corm. Moreover, sclereids were discovered in the closely related, but non-tussock forming, *E. viridicarinatum* Engelm. (Fern.) (Suspect ID). *E. viridicarinatum* (suspect) was also found in nutrient challenging and contaminated sites. Sclereids differ in this species from those of *E. vaginatum* in colour, hardness, and potentially in composition yet, position, size and morphology are similar. Sclereids in both species are found on the adaxial side of vascular bundles in cross-section. The role of sclereids and the mechanism of

*Eriophorum* spp. survival have not been determined. This study will elucidate the metal-sequestering ability of the sclereids in *E. vaginatum*, and to investigate for the presence of sclereids in other closely related species, i.e. within the Cyperaceae. We are currently examining the metal content of sclereids from *E. vaginatum* specimens of the Sudbury region, ON, by ICP-MS to investigate sclereids as a metal detoxification centre. This will determine sclereid potential as a plant survival mechanism in metal contaminated environments. Anatomical examinations of other species of the Cyperaceae, e.g. *Scirpus cyperinus* L. (Kunth.), and *Carex* spp., are ongoing to determine if sclereids are a species phenomenon. Sclereids were not found in the closely related *E. scheuchzeri* Hoppe., *S. hudsonianus* Michx., or *Carex paupercula Michx.* Results from this study may lead to the use of *E. vaginatum* as a bioremediative plant throughout the northern hemisphere.

## Poster Presentations:

**What is the role of extracellular calcium oxalate crystals in Araceae?**

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This study represents a survey of the pattern of production of extracellular calcium oxalate crystals in 15 genera of Araceae. Two methods were used to assess the presence of Extracellular crystals: (1) observation of floral structures at different stages of development, and (2) collection of pollen at dehiscence. For all Araceae observed using scanning electron microscopy (SEM), oxalate crystals exuding on the epidermal surface of the stamens correspond to extended aggregate/druses or crystal sand and the oxalate crystals mixed with pollen at dehiscence correspond to raphides or styloids (prismatic crystals). The type of crystals associated with pollen varies among genera. However, the presence or absence of crystals associated with pollen is a specific characteristic rather that a generic characteristic. Our results show that the presence of crystals mixed with pollen is correlated with the phylogeny of the family. For example, raphides mixed with pollen are the only crystals found on inflorescences with bisexual flowers. On the other hand, inflorescences with unisexual flowers (subfamily Aroideae) can have prismatic crystals and/or raphides.

**Analysis of *HUA2* gene Family in *Arabidopsis thaliana***

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Flowering, the transition from vegetative to reproductive growth is a major developmental switch in plants life cycle. Proper timing of this transition is crucial to ensure reproductive success, and is regulated by environmental cues and endogenous signals. The importance of this timing mechanism has become increasingly clear from genetic studies, which have revealed a large number of genes involved in regulation of flowering. *HUA2* is one of the genes that are identified from *Arabidopsis*, and acts as a floral repressor (Poduska et al, 2003; Qing et al., in preparation). The *HUA2* gene in Arabidopsis acts as a repressor of floral transition and belongs to a family of four genes, named *HUA2 L5*, *HUA2 L2*, and *HUA2 L3,* Fig.1. The *HUA2* gene, and members of the gene family have following putative motifs; PWWP, RPR, proline-rich region containing PPLP, and nuclear localization signals. HUA2-like proteins have not been identified in animal

species, indicating that this family of proteins may play roles unique to the plant development. My hypothesis is that *HUA2* homologues in *Arabidopsis* have redundant functions in plant development. The overall **objective** of my project is to investigate the functions of *HUA2* gene family in plant development.
As a first step to study the functions of the *HUA2* gene family, I investigated the developmental and tissue specific expression patterns in vegetative and reproductive tissues. Expression patterns of members of *HUA2* gene family are largely overlapping (Fig.2), suggesting possible functional redundancy. To test the function of each member of the *HUA2* family I am characterizing the T-DNA insertion lines in *HUA2 L5, HUA2 L2, HUA2 L3*. So far we have characterised RNA-nulls in *HUA2 L2* and *HUA2 L3*, but not in *HUA2 L5*, Fig.3. Promoter:GUS analysis is currently underway to determine the tissue specific expression pattern of each homologue, Fig.4. Creation of double, triple and multiple order mutants between *HUA2* and *HUA2* homologues will ultimately test functional redundancy among these genes.

**_De novo_ meristem generation in _Arabidopsis_ foliar explants**

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Many species of plants have a remarkable ability to generate meristems from somatic cells. To investigate this process at the molecular and cellular levels, we have developed a system to study shoot and root organogenesis from somatic cells in foliar explants of the model plant *Arabidopsis*. As an initial step in investigating this multi-gene process we have conducted a natural variation screen and characterized the *de novo* generation of shoot- and root meristems in 60 ecotypes. Two ecotypes (Nossen & Estland) with good shoot organogenic abilities (~100% of explants) and 3 ecotypes (Landsberg *erecta*, Dijon-G & Columbia) with low shoot regeneration (~0%) were selected for further investigation. Mapping has subsequently identified loci associated with the ability to regenerate shoots from cotyledon explants in some of these ecotypes. With regard to shoot- and root organogenesis and cell proliferation in the selected ecotypes, we have discovered critical periods of sensitivity to the application of exogenous hormones, the exposure to light and age of the explant tissues. Utilizing these critical conditions in specific combinations, we have conducted a mutant screen of 20,000 M2 explants to identify key genes that regulate shoot organogenesis in response to these conditions. From this screen and subsequent rescreening we have isolated 12 EMS mutants with consistently enhanced shoot organogenic abilities under normally non-permissive conditions. To examine to roles of various hormone-response and developmental pathways in *de novo* meristem generation, we are comparing organogenesis in ~100 previously characterized mutants with altered developmental, hormone- and light-responses. Further, we are utilizing a variety of GUS and GFP markers to dissect the developmental process in greater detail and to characterize the

effects of a variety of conditions on the different stages of shoot organogenesis. Additionally, we have studied the responses of the selected ecotypes to the exogenous application of various phytohormones and hormone response inhibitors. Guided by these observations we have begun to elucidate the mechanisms underlying the differing abilities of the ecotypes to generate *de novo* shoot meristems and identified treatments that promote shoot-organogenesis in normally recalcitrant ecotypes.

**Identification and characterization of the *Arabidopsis* exocyst complex**

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Vesicle trafficking is an important activity in plants that is central to a number of processes such as cell growth/expansion, pollen tube elongation and gravitropic response. Vesicles can travel intracellularly between compartments within the cell; vesicle trafficking can also include endocytosis, where cargo is taken in for processing from outside of the cell, and exocytosis, where products from inside the cell are brought to the plasma membrane or secreted to the cell exterior (reviewed in [1]). Molecular mechanisms of exocytosis have been extensively studied in yeast and mammals resulting in the identification of a multi-subunit complex called the exocyst. This complex is involved in the docking of exocytotic vesicles during polarized secretion. The yeast exocyst is composed of eight proteins: Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p (reviewed in [2]). Homologues for most of the exocyst complex members have been identified for number of animals, including Drosophlia [3, 4], humans and mouse (reviewed in [5]) and appear to be encoded by single copy genes. Study of the exocyst complex in plants is not as advanced as in animals. Recently a homologue of Sec3 was identified in corn whereby reduction in expression of this gene results in plants that with root hairs that cannot elongate [6]. Through bioinformatics analysis of the *Arabidopsis* genome, putative homologues for the exocyst complex have been identified. AtSec6, AtSec8, AtSec10 and AtExo84 appear to be single copy genes whereas putative AtSec3, AtSec5 and AtSec15 may be encoded by two genes. However, Exo70p in *Arabidopsis* appears to have diverged into a family of 23 members. Our primary focus is on AtExo70s. Analysis of microarray databases revealed that AtExo70s is widely expressed in *Arabidopsis.* Two independent T-DNA insertion lines have been identified for AtExo70s, and homozygotes from both lines show a similar phenotype. The plants are short and bushy and also initially appeared to be sterile. Knockout plants produce less pollen, but the sterility appears to be mainly attributed to inefficient delivery of pollen to the papillae where mature anthers never extend above the pistil. Further characterization of these knockout lines is currently underway. To investigate the role of AtExo70s in vesicle trafficking, the full length protein was fused to GFP and transiently expressed in tobacco BY-2 suspension cells. Preliminary results show that the fusion protein localized to distinct regions in the cell.

**'Prepackaged symbioses': propagules on roots of the myco-heterotrophic plant *Arachnitis uniflora.***

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*Arachnitis uniflora* (Corsiaceae) is a myco-heterotrophic, achlorophyllous plant species that is restricted in its distribution to Argentina, Chile, Bolivia, and the Falkland Islands. Plants are visible above ground only at the time of flowering. Each plant consists of several fleshy tuberous roots colonized by fungal hyphae, and a flower stalk that bears a single flower. The fungus present in the roots belongs to the genus *Glomus*, an arbuscular mycorrhiza genus in the ancient phylum Glomeromycota. Hyphal connections are formed between *A. uniflora* roots and the roots of surrounding photosynthetic plants through which carbon compounds needed for growth and reproduction are obtained. The fleshy roots produce propagules from their apices and from lateral sites along the root axis. The former are initiated immediately beneath the root cap and with development, a shoot apical meristem is initiated and the basal region of the propagule becomes colonized by fungal hyphae that likely originate from colonized cells in the parent root. Lateral propagules are initiated in cortical cells and these also develop a shoot meristem and become colonized by fungal hyphae. Propagules detach from the parent root, establishing new plants that are already colonized by an arbuscular mycorrhizal fungus. This method of vegetative propagation is unique to this species.

**Altered root development of the pea nodulation mutant R50 (*sym16*).**

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R50 is a pea mutant displaying short and thick basal internodes, decreased epicotyl height, and fewer and shorter lateral roots (LR). It has elevated levels of cytokinins (CK) because of deficient activity and/or altered expression of the enzyme CK oxidase/dehydrogenase. To characterize the R50 root phenotype, we studied the development of R50 root vasculature and compared it to that of Sparkle, the parent from which R50 was derived. Two sets of 18 plants were harvested at 4 different ages, and cross-sections of primary root (PR) were made by hand at three different locations. Two LR were also sectioned 1cm distal from their site of branching. Toluidine blue-stained sections were viewed by light microscopy and the number of xylem poles counted. Sparkle PR possessed 3 xylem poles, whereas the number of poles in R50 varied from 3 to 5 and often increased from tip to base. However, as R50 aged, more plants displayed the triarch vasculature. Surprisingly, LR vasculature differed from that of PR;

Sparkle LR had 3 to 4 poles but those of R50 had 2 to 3. The diameters of root and stele were recorded in another set of 18 plants and possible correlations were drawn. There was a consistent root/stele diameter ratio in Sparkle; however, the ratio was more variable in R50. No correlation was found between number of poles, stele diameter and root diameter. Finally, the numbers of initiated and emerged LR were determined in cleared root systems. There were less LR initiated and emerged in R50 than in Sparkle. Here, we have demonstrated that R50 root development is significantly different from that of Sparkle. Whereas some of our findings can be explained by the high CK levels of R50, others could be evidence for an auxin involvement in the R50 root phenotype.

**Functional Characterization of Selected Members of the Arabidopsis Plant U-Box ARM containing Family (AtPUBs)**

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In Brassica, the ARC1 (Arm Repeat Containing 1) protein has been shown to be a positive effector and a downstream component of the S-Receptor Kinase (SRK) in the self incompatibility response. Following the activation of ARC1 by SRK, it acts to promote the degradation of an unknown target via ubiquitination through its U-Box domain thus preventing self pollination. Arabidopsis contains 41 Plant U-Box proteins (AtPUBs) that based on sequence similarity are predicted to be related to ARC1, all of which contain a U-Box domain as well as a set of Arm repeat motifs1. Arm repeat motifs are commonly involved in protein-protein interactions, and in Brassica are involved in the interaction of ARC1 with SRK2. The U-Box domain has been characterized as a motif that confers E3 ubiquitin ligase activity on the protein3, indicating a role for these proteins in regulated protein proteolysis4,5. Our lab is interested in determining the function of selected members of this family in self compatible Arabidopsis. In particular we are interested in a subset of these AtPUBs that contain a more extensive Arm repeat region in comparison to other AtPUB members. To this end, we have screened for T-DNA insertions in these selected members, and have been characterizing those mutants that display a phenotype with a single insertion. This has included both overall phenotype analysis as well as germination assays under different conditions. In addition, yeast Two-hybrid assays have been conducted to determine whether these proteins may be involved in signaling pathways, acting downstream of selected Arabidopsis S-Domain kinases. We have also searched through the publicly available microarray databases to determine the expression of three of the AtPUB members under various stress conditions6. To investigate the potential role that these proteins may have in selective protein degradation, ubiquitination assays and subcellular localization studies are also currently underway.

**A reverse genetics approach to understanding mucilage synthesis and secretion in the *Arabidopsis* seed coat.**

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In *Arabidopsis*, differentiation of the epidermal cells of the seed coat after fertilization leads to the production of a pectinaceous mucilage, which is synthesized in the Golgi apparatus and secreted between the plasma membrane and the primary cell wall. Very little is known about which genes are involved in the synthesis and secretion of pectins and what roles they play. To address this, we are using reverse genetics to identify genes that may be involved in mucilage production in the epidermal cells of the *Arabidopsis* seed coat. To identify candidate genes, datamining was performed on families of genes expected to act in cell wall production and/or modification. In particular, genes were screened on the basis of their AtGenExpress microarray expression levels in developing seeds as visualized with eNortherns (Botany Array Resource, University of Toronto). Genes which showed high expression levels in seed and silique tissues were selected for this study. Knockout mutants for a number of these candidate genes are currently being isolated. To date we have examined the phenotype of 16 mutant lines, four of which showed obvious seed coat phenotypes. Two have slight defects and represent members of the pectin methyl esterase gene family, while the others have rather less mucilage and encode members of the cellulose synthase superfamily. More Salk T-DNA insertion lines from different gene families are currently in the process of being genotyped and examined. Through the study of these mutant lines we hope to refine the genetic pathway that is being developed for the regulation of mucilage production and secretion during seed coat epidermal cell differentiation.

**Confocal Imaging of Endodermal Cell Files Along the Onion Root Axis.**

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In most plant species, suberin lamellae are deposited as secondary walls in the endodermis. Some cells of this layer, however, do not develop these lamellae and are called "passage cells". They are usually situated near the xylem poles as seen in root cross sections. A longitudinal view is needed to determine whether passage cells are in continuous files, scattered, or more numerous in regions where lateral roots will be produced. Techniques are available to stain Casparian bands and suberin lamellae in cleared, whole roots. The present work is the first to provide a CLSM (confocal laser scanning microscope) procedure for suberin lamellae, allowing passage cells to be distinguished by their lack of staining. The

endodermis was exposed by treating roots with pectinase for 24 h, after which the central cortex and epidermis could be peeled away from the endodermis and stele. The suberin lamellae in the endodermis were stained with 0.01% Nile red dissolved in lactic acid. The solvent served to clear the tissue, resulting in a clearer visualization of the lamellae. A series of longitudinal optical sections of the endodermis were obtained using an LSM 510 META CLSM, and a three-dimensional reconstruction of the layer enabled passage cells to be distinguished from the endodermal cells with suberin lamellae. It was evident that the passage cells were scattered within the endodermis and were not concentrated in any one region. The physiological significance of this pattern will be to transfer water and ions such as calcium and magnesium to stele, as suberin lamellae deposition severely curtails the movement of these molecules.

***Phialocephala fortinii* opportunistically parasitizes epiphytic orchid seeds.**

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Orchid seeds are referred to as 'dust seeds' because of their small size. Seeds contain a minute undifferentiated embryo that lacks a shoot and root apical meristem. Because the seeds lack an endosperm, the limited storage reserves are confined to the embryo. In an environment lacking simple sugars, orchid seeds require a fungal symbiont for germination and the development of a structure called a protocorm. The usual fungal symbionts are basidiomycetes that are able to break down complex carbohydrates in the environment, supplying simple sugars to imbibed seeds for embryo growth and protocorm development. Phialocephala fortinii, a dark septate endophyte belonging to the hyphomycetes, is a root-inhabiting fungus which is capable of breaking down complex carbohydrates and proteins and is believed, in some cases, to be mycorrhizal. Previous investigations of the interactions between dark septate endophytes and orchids have been limited to terrestrial orchid roots. To investigate whether P. fortinii could trigger orchid seed germination and stimulate protocorm development, seeds of four epiphytic orchid species (Phalaenopsis amabelis, Cattleya jenmanii, Cattleya temuis, and Cattleya aclandiae) were cultured on plates containing a complex carbohydrate (ground oats), which were then inoculated with the fungus. Seeds were also cultured on control plates containing oats only, simple sugars only, and a mixture of the two. Seeds on media with simple sugar alone or sugar combined with oats had significantly higher germination rates than seeds on oats alone or oats plus fungus. Rather than functioning symbiotically, P. fortinii parasitized the seeds. Hyphae entered seeds through the micropylar region and spread throughout the seed, often digesting the entire embryo. This occurred in both viable and nonviable seeds. This is the first study to determine the effects of a dark septate fungal species on orchid seed germination. Results indicate that under certain circumstances, P. fortinii is opportunistic, utilizing any available carbon source, including living tissue.